Acute Encephalitis Syndrome

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Outline of the presentation

• What is AES?
• History of AES in India
• Infectious causes of AES in India
• Enhanced surveillance for AES in India
• Lessons learnt from enhanced surveillance
• AFI/AES surveillance
• Non infectious causes of AES in India
• Summary
Acute Encephalitis Syndrome (AES)

WHO Recommended case definition

- A person of any age, at any time of year with the acute onset of fever and a change in mental status (including symptoms such as confusion, disorientation, coma or inability to talk)

- AND/OR new onset of seizures (excluding simple febrile seizures*).

* A simple febrile seizure is defined as a seizure that occurs in a child aged 6 months to less than 6 years old, whose only finding is fever and a single generalized convulsion lasting less than 15 minutes, and who recovers consciousness within 60 minutes of the seizure.
Multiple Etiological Agents in AES

- Bacterial: Hib, Sp, Nm
- HSV, VZV
- Dengue
- JE
- WNV
- Measles & Mumps
- Enteroviruses
- HSV, VZV
- OTHERS
  - Autoimmune
  - Metabolic
  - Toxic
- Unknown?
Geographic expansion of JE in India

- Vellore District, 1955
- Bankura & Burdwan, 1973
- Uttar Pradesh, 1978, 2005
- Assam, 1978
- Delhi, 2011
- Bihar, 2000
- Jharkhand, 2011
- Tripura, 2013
- Bellary, 1986
- Andhra Pradesh, 1997
- Kerala, 1994
- Vellore District, 1955
- Courtesy: Dr P.K Sen, NVBDCP
Suspected JE Cases and Deaths in India

- No regular reporting system before 2005
- Routine sentinel site based surveillance after 2005

Courtesy- Dr P.K Sen, NVBDCP
Perception of AES in India:

AES = JE or other viral encephalitis

- Frequent outbreaks of acute encephalitis
  - Uttar Pradesh
  - Bihar
  - West Bengal
  - Assam
  - Andhra Pradesh
  - Tamil Nadu
  - Karnataka

- Overall Etiological diagnosis is established only in 10-12% of cases
NVBDCP Surveillance Data: Reported JE/AES Cases in India, 2008 – 2015

Source: NVBDCP
### Objectives of the study

1. **Work with state and national programs to establish a tiered network to support & strengthen laboratory based surveillance of JE/AES in India**
   - Strengthen district laboratory capacity for JE testing
   - Strengthen referral laboratory capacity for testing additional (non-JE) pathogens that may cause AES
   - Establish external quality assurance program with proficiency testing
   - Establish and enhance specimen transport and reporting of results

2. **Enhance the understanding of etiologies and epidemiology of AES in highly affected states**
   - Develop and use a standardized laboratory testing algorithm for JE & Non JE pathogens
   - Streamline data collection and reporting of results
     - Guide modification of routine surveillance
     - Develop appropriate public health and clinical intervention

### AES: Challenges

| **Clinical** | Case Identification and Classification:  
- AES is a broad umbrella term that can encompass a wide range of diagnoses |
| **Laboratory** | Lack of a Standard Testing Algorithm:  
- Routine district-level testing is primarily focused on JE  
- Lack of a standard laboratory algorithm to support diagnosis and identify treatable non-JE etiologies  
Laboratory Network with Robust Sample Referral System:  
- Strengthen existing district capacity and enhance linkages with referral level laboratories to support advanced diagnostic testing |
| **Epidemiologic** | Surveillance Data Flow:  
- Enhance existing data reporting and analysis for decision making |
| **Administrative** | Multiple stakeholders:  
- National program officers, state health authorities, district health officials, medical colleges, clinicians, nodal agencies. |
AES Surveillance: What did we do?

Step 1: Engaged with Stakeholders
NVBDCP, State health authorities, Medical colleges and district hospitals

Step 2: Identified sites and built a network
Standardized checklist to identify sites and linked districts to referral labs

Step 3: Finalized testing strategy
Consulted clinicians, microbiologists, state and central VBD officers, public health specialist

Step 4: Built Laboratory capacity
Trained - laboratories at district and referral level, public health workforce

Step 5: Established a sample transport mechanism
One key human resource at each site, specimen tracking and transportation

Step 6: Streamlined data collection and reporting
Uniform validated data collection tool, developed web based and android based data collection tool, reporting to all stakeholders at defined turn-around times
## Project Sites, 2014 – 2017

Site selection performed in consultation with national and state NVBDCP offices.

<table>
<thead>
<tr>
<th>State</th>
<th>Apex Laboratory</th>
<th>Districts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uttar Pradesh</td>
<td>King George Medical University</td>
<td>Deoria* Kushinagar* Siddharth Nagar Maharajganj Sitapur* Lakhimpur Kheri*</td>
</tr>
<tr>
<td>Assam</td>
<td>Assam Medical College</td>
<td>Dibrugarh* Jorhat Sibsagar Guwahati/Kamrup*</td>
</tr>
<tr>
<td>West Bengal</td>
<td>School of Tropical Medicine</td>
<td>Bankura Burdwan* Siliguri</td>
</tr>
<tr>
<td>Karnataka</td>
<td>NIMHANS</td>
<td>Bellary*</td>
</tr>
</tbody>
</table>

* Sites functional since 2014
Suspected AES case: Prompt CSF, blood and serum collection

**CSF**
- CSF cell count
- CSF biochemistry
- CSF JEV IgM Assay (ELISA) : Test at district hospital immediately and report results to clinicians and referral lab. If not possible send for immediate testing at referral lab.

**Blood/Serum**
- Hb, WBC count / differential, Platelets, Electrolytes, Glucose, LFT
- Malaria (Smear or RDT)
- JEV, Scrub Typhus and Dengue IgM Assay (ELISA). Test at district hospital immediately and report results to clinicians and referral lab. If not possible send for immediate testing at referral lab.

Send CSF and Serum aliquots promptly to referral lab for additional testing

**Molecular Tests (PCR)**
1. Bacterial PCR (Hib, Spn, Nm)
2. Herpesvirus DNA PCR
3. Enterovirus RNA PCR
4. Trioplex PCR (Zika, DENV, CHIKV)

**Serological Assays**
1. Dengue NS1
2. West Nile virus IgM
3. Chikungunya IgM
4. Leptospira IgM

**Molecular Tests (PCR)**
1. Trioplex PCR (Zika, DENV, CHIKV)

**DIAGNOSTIC CLASSIFICATION**
1. AES-JE  2. AES-DENGUE  3. AES-SCRUB TYPHUS  4. AES-WNV  5. AES-PYOGENIC MENINGITIS  
### Salient socio-demographic details of AES cases enrolled in the network over a four-year period (2014-17)

<table>
<thead>
<tr>
<th>State</th>
<th>Assam</th>
<th>UP</th>
<th>West Bengal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AES cases enrolled</td>
<td>5415</td>
<td>2797</td>
<td>1895</td>
<td>10107</td>
</tr>
<tr>
<td>Age range (in Years)</td>
<td>n=5409</td>
<td>n=2796</td>
<td>n=1652</td>
<td>n=9857</td>
</tr>
<tr>
<td>&lt;15</td>
<td>2756</td>
<td>2445</td>
<td>979</td>
<td>6180</td>
</tr>
<tr>
<td></td>
<td>(51%)</td>
<td>(87%)</td>
<td>(59%)</td>
<td>(63%)</td>
</tr>
<tr>
<td>Gender</td>
<td>n=5415</td>
<td>n=2797</td>
<td>n=1705</td>
<td>n=9917</td>
</tr>
<tr>
<td>Male</td>
<td>3308</td>
<td>1459</td>
<td>967</td>
<td>5734</td>
</tr>
<tr>
<td></td>
<td>(61%)</td>
<td>(52%)</td>
<td>(57%)</td>
<td>(58%)</td>
</tr>
<tr>
<td>Specimen collected</td>
<td>Assam</td>
<td>UP</td>
<td>West Bengal</td>
<td>Total</td>
</tr>
<tr>
<td>Serum &amp; CSF</td>
<td>2918</td>
<td>2141</td>
<td>875</td>
<td>5934</td>
</tr>
<tr>
<td></td>
<td>(54%)</td>
<td>(77%)</td>
<td>(46%)</td>
<td>(58%)</td>
</tr>
<tr>
<td>Serum</td>
<td>1629</td>
<td>474</td>
<td>394</td>
<td>2497</td>
</tr>
<tr>
<td></td>
<td>(30%)</td>
<td>(17%)</td>
<td>(21%)</td>
<td>(24%)</td>
</tr>
<tr>
<td>CSF</td>
<td>868</td>
<td>182</td>
<td>626</td>
<td>1676</td>
</tr>
<tr>
<td></td>
<td>(16%)</td>
<td>(7%)</td>
<td>(33%)</td>
<td>(17%)</td>
</tr>
<tr>
<td>Total</td>
<td>5415</td>
<td>2797</td>
<td>1895</td>
<td>10107</td>
</tr>
<tr>
<td>Mortality</td>
<td>605/4226</td>
<td>80/1920</td>
<td>263/1331</td>
<td>948/7477</td>
</tr>
<tr>
<td></td>
<td>(14%)</td>
<td>(4%)</td>
<td>(20%)</td>
<td>(13%)</td>
</tr>
</tbody>
</table>
Summary of AES Surveillance Network:
Jan 2014 - Dec 2017

AES Cases (n=10,107)

Antibody/Antigen based assays
- JEV IgM
  1641/10107 (16.23%)
- Scrub typhus IgM
  1155/7062 (16.36%)
- Dengue IgM/NS1*
  353/6650 (5.30%)
- West Nile IgM
  30/5662 (0.53%)
- CHIKV IgM*
  67/1402 (4.78%)
- Leptospira IgM*
  41/1600 (2.56%)

Nucleic acid based assays
- S.Pneumoniae PCR
  74/5158 (1.43%)
- N.meningitidis PCR
  3/5139 (0.06%)
- H.Influenzae PCR
  8/5036 (0.16%)
- Herpes Simplex PCR
  22/5299 (0.41%)
- Enterovirus PCR
  7/4608 (0.15%)
- Trioplex PCR* DENV+
  6/731 (1%)

Cumulative % of etiologies: 45.76%
Cumulative % of etiologies: 3.20%

- 45,315 AES cases reported to NVBDCP in 2014 – 2017
  - 31,092 AES cases from UP, Assam, WB, Karnataka (69% of total)
  - 10,107 AES cases tested represents 33% of reported AES cases in these states

Overall the application of testing algorithm resulted in etiologic identification in 49% of cases
  - JE, Scrub Typhus, Dengue account for almost 93% of these cases
### JEV, Scrub Typhus and Dengue Diagnoses, 2014 – October 2017

<table>
<thead>
<tr>
<th>Year</th>
<th>JE Diagnostic Testing Results</th>
<th>Assam</th>
<th>West Bengal</th>
<th>Uttar Pradesh</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>JE Diagnosis*</td>
<td>157/409 (38%)</td>
<td>47/190 (25%)</td>
<td>56/396 (14%)</td>
</tr>
<tr>
<td></td>
<td>Scrub typhus IgM+</td>
<td>22/162 (14%)</td>
<td>5/92 (5%)</td>
<td>191/403 (47%)</td>
</tr>
<tr>
<td></td>
<td>Dengue IgM+</td>
<td>2/149 (1%)</td>
<td>2/78 (3%)</td>
<td>27/456 (6%)</td>
</tr>
<tr>
<td>2015</td>
<td>JE Diagnosis*</td>
<td>315/1210 (26%)</td>
<td>53/351 (15%)</td>
<td>63/343 (18%)</td>
</tr>
<tr>
<td></td>
<td>Scrub typhus IgM+</td>
<td>71/509 (14%)</td>
<td>36/107 (34%)</td>
<td>127/465 (27%)</td>
</tr>
<tr>
<td></td>
<td>Dengue IgM+</td>
<td>16/238 (7%)</td>
<td>27/144 (19%)</td>
<td>18/526 (3%)</td>
</tr>
<tr>
<td>2016</td>
<td>JE Diagnosis*</td>
<td>287/1672 (17%)</td>
<td>77/473 (16%)</td>
<td>77/742 (10%)</td>
</tr>
<tr>
<td></td>
<td>Scrub typhus IgM+</td>
<td>93/1043 (9%)</td>
<td>34/100 (34%)</td>
<td>333/914 (36%)</td>
</tr>
<tr>
<td></td>
<td>Dengue IgM+</td>
<td>68/1044 (7%)</td>
<td>5/108 (5%)</td>
<td>73/1010 (7%)</td>
</tr>
<tr>
<td>2017**</td>
<td>JE Diagnosis*</td>
<td>460/1826 (25%)</td>
<td>66/557 (12%)</td>
<td>215/898 (24%)</td>
</tr>
<tr>
<td></td>
<td>Scrub typhus IgM+</td>
<td>50/1340 (4%)</td>
<td>97/298 (33%)</td>
<td>263/761 (35%)</td>
</tr>
<tr>
<td></td>
<td>Dengue IgM+</td>
<td>55/821 (7%)</td>
<td>31/303 (10%)</td>
<td>104/867 (12%)</td>
</tr>
</tbody>
</table>

*JE Diagnosis: CSF JEV IgM+ OR Serum JEV IgM+

**Among GHSA-NIMHANS Sites:**
- **Assam Sites:**
  - JE most common etiology
  - Scrub typhus less common
  - Dengue accounts for 10-15%
- **West Bengal Sites:**
  - JE accounts for ~12% of AES
  - Scrub typhus: prevalence >20%
  - Dengue notable
- **Uttar Pradesh Sites**
  - JE accounts for ≤ 10% AES
  - Scrub typhus prevalence >25%
  - Dengue: 4-8% of AES
Seasonality of AES in North India (2015-17)

Panel A: Seasonality of AES cases in Assam, Uttar Pradesh and West Bengal over a three year period (2015-2017)

Panel B: Seasonality of JE, Scrub Typhus and Dengue cases in Assam over a three year period (2015-2017)

Panel C: Seasonality of JE, Scrub Typhus and Dengue cases in Uttar Pradesh over a three year period (2015-2017)

Panel D: Seasonality of JE, Scrub Typhus and Dengue cases in West Bengal over a three year period (2015-2017)
Relative Risk factor study for Scrub typhus

- **Policy:**
  - Recognition of scrub typhus as an entity
  - Revision of National AES testing algorithm
  - Sustainability through NHM

- **Collateral spin-offs**
  - Zika surveillance system – GBS and AES
  - Scrub typhus risk factor study with ICMR, WHO, MCVR
  - AFI-AES combined surveillance with MCVR
  - Digital platform for data collection and reporting

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Adjusted Odds Ratio (95% CI)</th>
<th>Adjusted Population attributable fraction (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of house within/ adjoining field</td>
<td>1.59 (1.04-2.43)</td>
<td>15.2 (3.5-25.5)</td>
</tr>
<tr>
<td>Went for defecation in the field in the last 2 weeks (vs toilet or around house)</td>
<td>2.20 (1.39-3.48)</td>
<td>14.4 (8.7-19.8)</td>
</tr>
<tr>
<td>Visited agriculture field in the last 2 weeks</td>
<td>1.66 (1.09-2.52)</td>
<td>9.2 (3.2-14.9)</td>
</tr>
<tr>
<td>Storage of firewood inside house/verandah</td>
<td>1.59 (1.05-2.41)</td>
<td>7.8 (2.1-13.2)</td>
</tr>
<tr>
<td>Playing in the field in the last 2 weeks (vs indoor or around house)</td>
<td>2.11 (1.12-3.97)</td>
<td>2.1 (0.6-6.2)</td>
</tr>
<tr>
<td>Fed cattle in the last 2 weeks</td>
<td>1.87 (1.08-3.26)</td>
<td>-5.0</td>
</tr>
<tr>
<td>Bathing in river/nullah</td>
<td>1.73 (0.80-3.79)</td>
<td>5.0 (0-12.2)</td>
</tr>
</tbody>
</table>

Accepted for publication in *Emerging Infections Diseases* (Nov 2018)
Geographic Distribution of Scrub Typhus

From: Scrub Typhus: The Geographic Distribution of Phenotypic and Genotypic Variants of Orientia tsutsugamushi
AES Surveillance Health Informatics Platform

- Inter-linked Web-based and Android App-based health informatics platforms developed for AES surveillance network
  - In collaboration with Health Information Systems (HISP) India
  - Utilizes District Health Information Systems 2 (DHIS2) platform, already in use by NVBDCP
- Real-time data entry, management, analytics, and reports
- SMS alerts sent to treating physicians and site coordinators if test result is positive
Etiology of Pediatric AES-Bangalore (n=108)
Real-time Sentinel Infectious Disease Surveillance in India, 2014 – 2016

- Surveillance of 40,000 patients admitted to district and sub-district hospitals with acute febrile illness and/or acute encephalitis syndrome
- Systematic laboratory testing resulted in etiologic diagnosis in 40%
- Top 7 pathogens identified:
  - Dengue
  - Influenza
  - Japanese Encephalitis
  - Kyasnur Forest Disease
  - Leptospirosis
  - Malaria
  - Scrub Typhus
AFI/AES overlapping surveillance: Study design

• Prospective case control design
• Initiated from 1st August 2018 - Ongoing until 31st Dec 2018
• Two sites were selected – One PHC at Rampur Karkhana and One District Hospital at Deoria town.
• Standard AFI and AES case definitions
• Serum samples at PHC tested using Rapid diagnostic tests and subsequently transported same day to District hospital laboratory for confirmation by ELISA. Throat swab, urine stool samples transported to MCVR, Manipal
• Testing strategies used were identical to AFI/AES surveillance carried out earlier by MCVR and NIMHANS respectively
## Interim results

### Etiology identified on PHC patients

<table>
<thead>
<tr>
<th>Etiology identified</th>
<th>AFI (n=311)</th>
<th>AFI with altered mental status/seizure</th>
<th>Total (n=329)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHIK</td>
<td>5 (1.6%)</td>
<td>0</td>
<td>5 (1.5%)</td>
</tr>
<tr>
<td>DEN</td>
<td>27 (8.7%)</td>
<td>0</td>
<td>27 (8.2%)</td>
</tr>
<tr>
<td>DEN (IgM+NS1)</td>
<td>4 (1.3%)</td>
<td>0</td>
<td>4 (1.2%)</td>
</tr>
<tr>
<td>DEN IgM+CHIK</td>
<td>15 (4.8%)</td>
<td>0</td>
<td>15 (4.6%)</td>
</tr>
<tr>
<td>DEN IgM+LEPT</td>
<td>2 (0.6%)</td>
<td>0</td>
<td>2 (0.6%)</td>
</tr>
<tr>
<td>DEN IgM+LEPT+CHIK</td>
<td>1 (0.3%)</td>
<td>0</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>DEN IgM+ST</td>
<td>3 (1%)</td>
<td>0</td>
<td>3 (0.9%)</td>
</tr>
<tr>
<td>DEN IgM+ST+CHIK</td>
<td>4 (1.3%)</td>
<td>0</td>
<td>4 (1.2%)</td>
</tr>
<tr>
<td>DEN NS1+ST</td>
<td>2 (0.6%)</td>
<td>0</td>
<td>2 (0.6%)</td>
</tr>
<tr>
<td>DEN NS1+ST+LEPT+CHIK</td>
<td>1 (0.3%)</td>
<td>0</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>LEPT</td>
<td>11 (3.5%)</td>
<td>1 (5.6%)</td>
<td>12 (3.6%)</td>
</tr>
<tr>
<td>LEPT+CHIK</td>
<td>1 (0.3%)</td>
<td>0</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>NS1</td>
<td>3 (1%)</td>
<td>0</td>
<td>3 (1%)</td>
</tr>
<tr>
<td>ST</td>
<td>30 (9.6%)</td>
<td>1 (5.6%)</td>
<td>31 (9.4%)</td>
</tr>
<tr>
<td>ST+CHIK</td>
<td>1 (0.3%)</td>
<td>0</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>ST+LEPT</td>
<td>2 (0.6%)</td>
<td>0</td>
<td>2 (0.6%)</td>
</tr>
<tr>
<td><strong>Grand Total</strong></td>
<td><strong>112 (36%)</strong></td>
<td><strong>3 (16.7%)</strong></td>
<td><strong>115 (35%)</strong></td>
</tr>
</tbody>
</table>

### Etiology identified on District hospital patients

<table>
<thead>
<tr>
<th>Etiology identified</th>
<th>AFI (n=227)</th>
<th>AFI with altered mental status/seizures (n=266)</th>
<th>Total (n=493)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHIK</td>
<td>2 (0.9%)</td>
<td>4 (1.5%)</td>
<td>6 (1.2%)</td>
</tr>
<tr>
<td>DEN</td>
<td>16 (7%)</td>
<td>15 (5.6%)</td>
<td>31 (6.3%)</td>
</tr>
<tr>
<td>DEN(IgM+NS1)+LEP</td>
<td>0</td>
<td>1 (0.4%)</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>DEN(IgM+NS1)+ST+CHIK</td>
<td>2 (0.9%)</td>
<td>0</td>
<td>2 (0.4%)</td>
</tr>
<tr>
<td>DEN(IgM+NS1)</td>
<td>2 (0.9%)</td>
<td>0</td>
<td>2 (0.4%)</td>
</tr>
<tr>
<td>DEN(IgM+NS1)+WNV</td>
<td>1 (0.4%)</td>
<td>0</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>DEN+CHIK</td>
<td>8 (3.5%)</td>
<td>5 (1.9%)</td>
<td>13 (2.6%)</td>
</tr>
<tr>
<td>DEN+ST</td>
<td>3 (1.3%)</td>
<td>9 (3.4%)</td>
<td>12 (2.4%)</td>
</tr>
<tr>
<td>DEN+ST+CHIK</td>
<td>4 (1.8%)</td>
<td>5 (1.9%)</td>
<td>9 (1.8%)</td>
</tr>
<tr>
<td>LEP</td>
<td>2 (0.9%)</td>
<td>2 (0.8%)</td>
<td>4 (0.8%)</td>
</tr>
<tr>
<td>LEP+CHIK</td>
<td>1 (0.4%)</td>
<td>0</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>LEP+WNV+CHIK</td>
<td>1 (0.4%)</td>
<td>0</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>NS1</td>
<td>0</td>
<td>4 (1.5%)</td>
<td>4 (0.8%)</td>
</tr>
<tr>
<td>NS1+ST</td>
<td>2 (0.9%)</td>
<td>4 (1.5%)</td>
<td>6 (1.2%)</td>
</tr>
<tr>
<td>NS1+ST+LEP</td>
<td>0</td>
<td>1 (0.4%)</td>
<td>1 (0.2%)</td>
</tr>
<tr>
<td>ST</td>
<td>57 (25.1%)</td>
<td>89 (33.5%)</td>
<td>146 (29.6%)</td>
</tr>
<tr>
<td>ST+CHIK</td>
<td>6 (2.6%)</td>
<td>5 (1.9%)</td>
<td>11 (2.2%)</td>
</tr>
<tr>
<td>ST+LEPT</td>
<td>1 (0.4%)</td>
<td>1 (0.4%)</td>
<td>2 (0.4%)</td>
</tr>
<tr>
<td>WNV+CHIK</td>
<td>2 (0.9%)</td>
<td>0</td>
<td>2 (0.4%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>110 (48.5%)</strong></td>
<td><strong>145 (54.5%)</strong></td>
<td><strong>255 (51.7%)</strong></td>
</tr>
</tbody>
</table>
Interim summary of overlapping surveillance

- The etiological pattern of AFI between the PHC and district hospital were quite distinct
- District hospital at Deoria town has a large catchment area for patients as it caters to several other PHCs (16) & CHCs (8)
- Etiological agents were identified in 35% of AFI cases at Rampur Karkhana PHC (Influenza results are pending). Amongst those with an etiology Scrub typhus (9.6%), Dengue (8.7%), Chikungunya (1.6%) and leptospirosis (3.5%) accounted for 65% of cases.
- Etiological agents were identified in 48% of AFI cases at Deoria district hospital (Influenza results are pending). Amongst those with an etiology Scrub typhus (25%), Dengue (7%), Chikungunya (1%) and leptospirosis (1%) accounted for 70% of cases.
- Etiological agents were identified in 54% of AES cases enrolled at Deoria district hospital. Amongst those with an etiology, Scrub typhus (33.5%), Dengue (5.5%), Chikungunya (1.5%) and Leptospirosis (1%) accounted for 75% of all cases.
- The number of AES cases reported from Rampur Karkhana in 2018 were only 11/266 (4%) cases. In contrast the number of AES cases reported form the same block between 2015 & 2016 were totally 74/859 (9%). This clearly indicates that there is 50% reduction in AES because of initiating AFI surveillance at this PHC. Further, all the 11 cases 2018 did not report with fever to the PHC before presenting as AES directly to Deoria hospital.
Nipah virus: Anatomy of the 2018 outbreak

- May 17th, 2 A.M: 28 year/M, architect Mohd Salih rushed to Kozhikode’s Baby Memorial Hospital
- High grade fever, vomiting, altered sensorium (agitation)
- Heart rate: 180/minute; hypertensive
- ↓ reflexes.

Differential diagnosis considered
- R/O Japanese encephalitis (typically doesn’t affect more than one person in a household);
- His younger brother Mohd. Sabith died about 12 days ago after showing similar symptoms (patient zero), Rabies, Toxin mediated (R/O)
- Father and aunt, too, contracted the infection and later succumbed
Non-Infectious causes of AES identified in India

- Toxins
- Metabolic encephalopathy
- Autoimmune encephalitis
‘Saharanpur’ Encephalitis (Western UP)

- Outbreaks of unexplained ‘encephalitis’ in children every year-2 decades; Very high mortality (70-80%)
- Not encephalitis, but a multi-system disease affecting liver, muscle and brain
- Re-named “acute hepatomyoencephalopathy (HME) syndrome” caused by phytotoxins.
- Source of toxicity was found to be to the consumption of beans of a ubiquitous weed *Cassia occidentalis* by young children of poor families
- Cases occurred only in September-December every year coinciding with the poding season of this annual plant.
- 2008-2012 Shaharanpur, UP, 2017 Malkangiri, Orissa
AES in Muzaffarpur, Bihar
What was known from 2014 study

- Annual seasonal outbreaks during the months of April–July, 2 decades
- Affects hundreds of children; 40–60% mortality
- Coincided spatially and temporally with lychee cultivation & harvest
- Typical Clinical features: sudden onset without prodromal phase, inconsistent presence of fever, brain oedema, acellular CSF and hypoglycaemia
- Sparing of children below 2
- Children well until evening, but early next morning found seriously ill with brain function derangement and seizures.
- Consistent association- Malnourished children
- Toxic encephalopathy- Lychee contains toxin MCPG- causes hypoglycemia
- Well-nourished children not affected since their glycogen/glucose store in the liver is sufficient to maintain normal glucose levels
- Similar outbreak in litchi growing areas in Malda District (West Bengal)-June 2014
### Current status of AES outbreak

<table>
<thead>
<tr>
<th></th>
<th>SKMCH</th>
<th>KDKMH</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>New admissions on 20 June</td>
<td>26</td>
<td>5</td>
<td>31</td>
</tr>
<tr>
<td>Deaths on 26 June</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total AES cases till date</td>
<td>398</td>
<td>154</td>
<td>552</td>
</tr>
<tr>
<td>Total deaths</td>
<td>98</td>
<td>20</td>
<td>118 (21.4%)</td>
</tr>
</tbody>
</table>

#### Cases of AES by date of hospitalization, Muzaffarpur area, Bihar, 2019

![Cases of AES by date of hospitalization](chart.png)
Figure 1. Conversion of MCPG and hypoglycine into active metabolites and their sites of inhibition of oxidation16.
### Clinical Findings – No Inflammation

<table>
<thead>
<tr>
<th>Clinical Characteristics</th>
<th>N=390</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>31%   (121/390)</td>
</tr>
<tr>
<td>Temperature on admission &lt;99.5°F</td>
<td>61%   (219/359)</td>
</tr>
<tr>
<td>Time of illness onset between 3am – 8am</td>
<td>66%   (257/390)</td>
</tr>
<tr>
<td>Blood glucose on presentation &lt;70 mg/dL (hypoglycaemia)</td>
<td>62%   (204/327)</td>
</tr>
<tr>
<td>CSF examination with WBC &lt; 5 cells/mm³</td>
<td>84%   (52/62)</td>
</tr>
<tr>
<td>Brain MRI with no focal lesions</td>
<td>100%  (16/16)</td>
</tr>
<tr>
<td>EEG with generalized encephalopathy</td>
<td>100%  (30/30)</td>
</tr>
</tbody>
</table>
# Case Control Study -- Matched Bivariate Analysis

<table>
<thead>
<tr>
<th>KEY RISK FACTORS</th>
<th>CASES (N=104)</th>
<th>CONTROLS (N=208)</th>
<th>mOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ate litchi*</td>
<td>65% (67/103)</td>
<td>48% (98/204)</td>
<td><strong>2.1</strong> (1.2 – 3.5)</td>
</tr>
<tr>
<td>Ate rotten litchi*</td>
<td>26% (23/88)</td>
<td>15% (19/130)</td>
<td>2.4 (1.0 – 5.5)</td>
</tr>
<tr>
<td>Visited fruit orchard*</td>
<td>52% (52/100)</td>
<td>32% (62/195)</td>
<td><strong>2.9</strong> (1.6 – 5.1)</td>
</tr>
<tr>
<td>Parent visited fruit orchard*</td>
<td>31% (29/95)</td>
<td>20% (39/198)</td>
<td>1.8 (1.0 – 3.1)</td>
</tr>
<tr>
<td>Last meal before 6pm*</td>
<td>55% (54/98)</td>
<td>36% (63/176)</td>
<td><strong>2.0</strong> (1.2 – 3.2)</td>
</tr>
<tr>
<td>Higher SES**</td>
<td>8% (8/102)</td>
<td>16% (33/207)</td>
<td><strong>0.4</strong> (0.2 – 0.9)</td>
</tr>
<tr>
<td>Routinely wash vegetables and fruits</td>
<td>32% (32/99)</td>
<td>56% (102/183)</td>
<td><strong>0.32</strong> (0.2 – 0.6)</td>
</tr>
</tbody>
</table>

* 24 hours before illness  
** SES : Socio -Economic Status
Effect of Eating Litchi Modified by Absence of Evening Meal

<table>
<thead>
<tr>
<th>Last meal BEFORE 6pm</th>
<th>Last meal AFTER 6pm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposure</td>
<td>mOR (95% CI)</td>
</tr>
<tr>
<td>Ate litchis**</td>
<td>9.8 (0.6 – 159.6)</td>
</tr>
</tbody>
</table>

* Controlling for SES and routinely washing vegetables and fruits
** 24 hours before illness

Muzaffarpur outbreak illness is an acute hypoglycemic encephalopathy due to toxicity from MCPG/Hypoglycin in litchis exacerbated by the absence of eating an evening meal.
Investigations carried out in the Metabolic Laboratory, NIMHANS, Bengaluru

Free carnitine (C0) and a panel of 30 acylcarnitines (C2-C18) by Tandem Mass Spectrometry

Blood samples presumably collected before treatment

1. Plasma samples

No of samples tested : 33
No of cases with abnormalities : 25

(75.75%)

Types of abnormalities observed:

Decreased free carnitine: 8 cases
Decreased free carnitine with elevated acylcarnitines: 8 cases
Elevated acylcarnitines (short, medium & long-chain) : 9 cases
Blood acylcarnitines in samples before & after treatment

- C2, C5, C6, C12, C12, C12:1, C14, C14:1, C14, C14:1, C16, C16OH, C18, C18:1

- C2, C5, C6, C8, C5DC, C10, C10:1, C12, C12, C12:1, C14, C14:1, C14, C14:1, C16, C16OH, C18, C18:1

**Sample 1**
- C14:1

**Sample 2**
- C14:1

(20759) No. of abnormal analytes

(20523) No. of abnormal analytes
Carnitine

Quaternary amine essential for **transfer of long-chain fatty acids across the inner mitochondrial membrane** for subsequent β-oxidation

Causes of **low plasma free carnitine**

1. **Primary carnitine deficiency**
   Deficiency in carnitine transporter (OCTN2)

2. **Secondary carnitine deficiency**
   
   i) Loss of carnitine in the urine
      Organic acids in blood: mono/dicarboxylic acids, methylenecyclopropylformyl Co-A (MCPF-Co-A)
      Medication: valproic acid, pivalic acid, etc
      Fanconi syndrome
      Toxins-MCPG
   
   ii) Severe undernutrition
Anuska Kumari 7/F; Reg No. 20023

Ref by: Dr. Arun Singh, Professor Neonatology, National Advisor, RBSK MoHFW, Govt. of India
NIMHANS Ref: Npath No. X-3722/19 ; EM No. 187/19

Histochemical stains on skeletal muscle

H&E
MGT
SDH
NADH

COX_SDH
ATP 9.5
PAS
ORO

Histochemical stains shows mild fiber size variation
No evidence of mitochondrial pathology or storage material (glycogen, lipid)
**Respiratory chain enzyme assay on skeletal muscle**

<table>
<thead>
<tr>
<th>Test name</th>
<th>Result</th>
<th>% of control mean</th>
<th>Reference range</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I</td>
<td>0.08</td>
<td>27.89</td>
<td>0.107-0.499 mean= 0.30</td>
<td>μmols DCIP reduced.min⁻¹ mg protein⁻¹.unit citrate synthase⁻¹</td>
</tr>
<tr>
<td>Complex II</td>
<td>0.32</td>
<td>77.03</td>
<td>0.227-0.649 mean=0.42</td>
<td>μmols DCIP reduced.min⁻¹ mg protein⁻¹.unit citrate synthase⁻¹</td>
</tr>
<tr>
<td>Complex III</td>
<td>0.73</td>
<td>163.39</td>
<td>0.161-0.609 mean=0.45</td>
<td>μmols Cytochrome C reduced.min⁻¹ mg protein⁻¹.unit citrate synthase⁻¹</td>
</tr>
<tr>
<td>Complex IV</td>
<td>0.71</td>
<td>69.51</td>
<td>0.264-1.78 mean=1.02</td>
<td>μmols Cytochrome C oxidised.min⁻¹ mg protein⁻¹.unit citrate synthase⁻¹</td>
</tr>
</tbody>
</table>

**Conclusion:** The respiratory chain activity of Complex I is <30%
Electron micrographs of skeletal muscle

Ultrastructurally, skeletal muscle tissue shows a few enlarged mitochondria with altered cristae pattern and presence of electron dense material, presence of myeloid structures and distortion of myofilamentous pattern in a few fibers.
Neuromuscular Lab

Autoimmune encephalitis mosaic -332/2778 (11.95%) positive

- NMDA -251 (75.6%)
- LGI -25 (7.5%),
- CASPR -27 (8.1%),
- GABA -5 (1.5%),
- GAD -24 (7.2%)
AES: From Admission to Discharge

- **JE Encephalitis**
  - Confirmed JE (CSF IgM +)
  - Probable JE (Serum IgM+)

- **AES -- Other Identified Etiology**
  - Pyogenic meningitis
  - Other viral encephalitis: HSV, Dengue, WNV, CHIKV

- **Other etiologies of febrile illness**
  - Other febrile illness pathogen: Scrub typhus, malaria, Zika
  - Metabolic imbalance, Toxin

- **AES - Unknown**
  - **Pathogen Discovery Testing**

Time to revisit the nomenclature and use of the term AES?
OVERALL SUMMARY

Changing Scenario of AES in India

| Emergence of Pathogen in a New Geographic Area | • JE, DENGUE, Chikungunya |
| Emergence / Recognition of New Pathogen | • NIPAH, • SCRUB TYPHUS |
| Changes in Health Care | • “Opportunistic” encephalitides in immunosuppressed • JC/CMV/HSV/HHV-6 |
| Recognition of “new” etiologies of encephalitis | • Anti-synaptic receptor antibody mediated encephalitis • Toxin mediated encephalopathies |

- The epidemiology of AES in India is changing
- Etiological diagnosis of AES is best achieved using standard testing algorithms
- Identification of treatable causes of AES should get preference in the algorithm
- AES is only an admission diagnosis and it cannot be a discharge diagnosis
- Strengthening laboratory networks and establishing robust sample referral mechanisms are essential for tackling outbreaks
- Preventive strategies need to be evidence based.
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Flora and fauna of NIMHANS campus